12.0 GUIDANCE FOR PERFORMING BIOACCUMULATION TESTS

Bioaccumulation is defined in relation to disposal activities in the Definitions section at the beginning of this manual.

12.1 Tier III: Determination Of Bioavailability

Bioavailability tests are designed to evaluate the potential of benthic organisms to bioaccumulate contaminants of concern from the proposed dredged material. Lee et al. (1989) and Boese and Lee (1992) discuss bioaccumulation methodology in detail and may be followed on any matter that does not conflict with this manual. Tier III bioavailability tests are based on analysis of tissues of organisms after 28 d of exposure (see Section 6.3). Although time series testing is a component of Tier IV bioaccumulation testing, it may also be appropriate in Tier III, for instance where K_{ow} values are greater than 5.5 (see Section 12.2.1).

12.1.1 Species Selection and Apparatus

The selection of aquatic organisms for use in the determination of bioaccumulation will depend on their inability to metabolize some types of organic compounds, and their ability to survive exposure to the test sediments. Two species should be used in bioaccumulation testing where possible (Table 12-1), unless adequate regional data are available to justify single species testing. Test species should provide adequate biomass for chemical analysis, and preferably ingest sediments and survive in dredged material and control and reference sediments equally well (or where differences can be accounted for). The rationale for testing more than a single species is to cover the range of differing species contaminant accumulation and to be environmentally protective. Of the species tested, at least one must be a benchmark species; however, this does not preclude the use of more than one benchmark species. Non-benchmark species listed in Table 12-1 can achieve benchmark status if a summary of test conditions and test acceptability criteria similar to the starred benchmark species are provided that meet the required species characteristics criteria. To be technically justified, species proposed for use regionally and not listed in Table 12-1 would also need to meet the species characteristics criteria and proponents should provide a summary of test conditions and test acceptability criteria except where species are to be tested *in addition to* the benchmark species. In this latter case, this information is desirable but not needed.

Table 12-1. Candidate Test Species for Determining Potential Bioaccumulation from Whole Sediment Tests. Details of testing procedures are provided in Appendix E; additional guidance is provided in EPA (1994c,d).

Polychaetes

*Neanthes arenaceodentata** (N)
*Nereis virens** (N,E)[a]
Arenicola marina (N)

Oligochaetes

Lumbriculus variegatus (F)*

Insect Larvae

Mayfly, *Hexagenia limbata* or sp. (F)

Bivalves

Macoma clam, *Macoma nasuta**(N,E)[a]
Yoldia clam, *Yoldia limatula* (N)

Crustaceans

Diporeia sp. (F)

Note: Examples are not presented in order of importance; however, the asterisks indicate recommended benchmark species. Other species may be designated in future as benchmark species by EPA and USACE when the data on their response to contaminants are adequate. Only benthic species should be tested. Although sediment ingesters are preferable, intimate contact with sediment is acceptable.

Only tests which do not require feeding of the organisms are included. Feeding is a research issue; for the present, food is not to be added because it provides additional organic carbon and can alter contaminant partitioning during testing.

For the purpose of this manual, related to the tolerances of the test animals, (F) = Freshwater, salinity ≤ 1‰ (N) = Near Coastal, salinity ≥ 25‰ (E) = Estuarine, salinity 1-25‰. It is recognized that the commonly accepted salinity range for estuaries is 1-35‰ and near coastal water is usually greater than 30‰ salinity.

[a] *Macoma nasuta* and *Nereis virens* bioaccumulation tests are in the process of standardization by EPA; it is expected that these will, in future, be the primary benchmark species for near coastal waters. Further, these two species can be used in estuarine waters down to appropriate low levels of salinity (see Appendix E).

Apparatus to be used for testing is described in Section 11.2.2. Additional requirements for voiding gut contents are described in Section 12.1.2. Species characteristics to consider when designing bio-accumulation tests include, not in order of importance:

- readily available year-round
- provide adequate biomass for analysis
- preferably ingest sediments
- preferably high in lipids
- survive in dredged material and control and reference sediments equally well, allowing adequate tissue for analysis
- tolerate handling and laboratory conditions
- related phylogenetically and/or by ecological requirements to species characteristic of the disposal site area in the season of the proposed discharge
- important ecologically, economically, and/or recreationally
- inefficient metabolizers of contaminants, particularly PAH.

Regional scientists and regulatory personnel should be consulted for additional guidance. A minimum amount of tissue is required for analysis, otherwise it will be impossible to quantify the amount of contaminant present (Section 9.5.2). Examples of the amounts of tissue which may be required are provided in Table 8-2. However, the amounts shown are not set amounts; more or less may be required depending on the analytes, matrices, detection limits, and particular analytical laboratory. If the biological needs of the organisms or adequate voiding (e.g., clams) require the presence of sediment, uncontaminated sand should be used (Section 12.1.2). Data in the form of "concentration below detection limits" are not quantitative; definitive concentration measurements are the goal, where such are possible within reasonable method and target detection limits.

12.1.2 Experimental Conditions

Test conditions are similar but not identical to those described in Section 11.2.2 for whole sediment toxicity tests. Overlying water renewal may be required to maintain adequate water quality. Food or additional sediment should not be provided during the test. Control animals should be sampled and archived at both the beginning and the end of testing. If discrepancies are found during data analysis, the archived samples can be analyzed to possibly resolve any problem(s). Due care should be taken not to exceed species-specific biomass loadings (overcrowding; APHA, 1989).

Digestive tracts of the animals should be emptied or removed immediately after termination of the exposure period. Sediment in digestive tracts may contain inert constituents and the contaminants of concern in forms which are not biologically available but which may be incorrectly identified as such during chemical analysis (e.g., see Lobel et al., 1991).

If the animals are large enough to make it practical, the best procedure is to excise the digestive tract. However, test organisms are seldom large enough to allow this, and most organisms have to be allowed to void the material, in separate aquaria in clean, sediment-free water. Some organisms will pass material through the digestive tract only if more material is ingested. These animals have to be purged in aquaria with clean sand. Animals are not fed during the purging period. Fecal material is siphoned from the aquaria twice during the 24-h purging period. To minimize the possibility of loss of contaminants from tissues, purging for longer periods is not recommended. Shells or exoskeletons which generally contain low levels of contaminants are, where possible, removed and not included in the analysis as their weight would give an artificially low indication of bioavailability.

An initial time-zero of each sample is collected for tissue analysis. Tissue contaminant concentrations in control animals must be determined to ensure that background levels are not inordinate. Although procedures for Tier III and IV laboratory bioaccumulation tests have been discussed separately, it may be possible to combine these procedures in practice. This can be done by following the steady state (Tier IV) bioaccumulation procedure which involves sequential time-series analyses, but initially analyzing only the 28 d sample and freezing the other samples. If these data, as part of the Tier III bioavailability evaluation, do not allow a determination to be made, then the remaining time series samples may be analyzed and used in the Tier IV steady-state bioaccumulation evaluation.

12.1.3 Chemical Analysis

Chemical analysis will involve some or all of the contaminants identified in Sections 4.2 and 9.5.1. Analytical procedures are provided in Section 9.5.2.

12.1.4 Data Presentation and Analysis

Data Presentation

Data should be presented in tabular format, listing tissue concentration of each contaminant, by organism and by sediment type (e.g., dredged and reference). Similar information to that detailed in Section 11.2.4

should be provided. Although bioaccumulation species/tests cannot be used to determine toxicity requirements, any mortalities which occur during bioaccumulation testing must be documented.

Data Analysis

Contaminant tissue concentrations in test organisms are statistically compared to the FDA Action Levels (Table 6-1) (refer to Figure 3-3). These tissue concentrations are also statistically compared with reference organism concentrations (Appendix D). In some cases, tissue concentrations in organisms exposed to one or more of the dredged-material samples may be less than or equal to reference organism concentrations. Providing the reference data are appropriate, this result indicates that bioavailability of the contaminants of concern in the dredged material is not greater than in the reference area sediment.

The sample of organisms archived at the initiation of the exposure can be useful in interpreting results. It can add perspective to the magnitude of uptake during the exposure period. In some cases, elevated body burdens may not be due to the dredged material or reference sediment, but may have been already present in the organisms at the start of the test.

12.1.5 Conclusions

Guidance on reaching a determination is provided in Section 6.3.

12.2 Tier IV: Determination Of Steady State Bioaccumulation

Tier IV bioaccumulation evaluation, if necessary, provides for determination, either by laboratory testing (ASTM, 1984) or by collection of field samples, of the steady state concentrations of contaminants in organisms exposed to the dredged material as compared with organisms exposed to the reference site material. Testing options include longer laboratory exposures (not discussed), collection of organisms living in the material to be dredged and at the reference site for body burden determinations (Section 12.2.2) or *in situ* exposures using transplanted organisms, for instance caged mussels (not discussed). Tier IV determinations follow the guidance in Section 7.2.

12.2.1 Laboratory Testing

The necessary species, apparatus and test conditions for laboratory testing are those for Tier III bioaccumulation testing (see Sections 12.1.1 and 12.1.2). Tissue samples taken at different times during

the exposure period provide the basis for determining the rate of uptake and elimination of contaminants. From these rate data, the steady state concentration of contaminants in the tissues can be calculated, even though the steady state might not have been reached during the actual exposure. For the purposes of this test, steady state is defined as the concentration of contaminant that would occur in tissue after constant exposure conditions.

An initial time-zero sample of each species is collected for tissue analysis. Additional tissue samples are collected from each of the five replicate reference and dredged-material exposure chambers at intervals of, for instance, 2, 4, 7, 10, 18, and 28 d. It is critical that enough tissue is available to allow for interval body burden analyses at the specified detection limits.

Complete tissue concentration data should be presented in tabular format. Recommended statistical methods for fitting a curve to determine steady-state tissue concentration are provided in Appendix D. The statistical procedures use an iterative curve-fitting process to determine the key variables ($k_1 C_s$ the uptake rate-constant times the contaminant concentration in the sediment, and k_2 the depuration rate constant). An initial value for C_s has to be supplied. When the sediment concentration of the contaminant of concern is used, the ratio of k_1/k_2 is the sediment bioaccumulation factor (BAF) (Lake et al., 1987; Rubinstein et al., 1987), the ratio of steady-state tissue concentration to sediment concentration.

A determination is made based on the magnitude of bioaccumulation from the dredged material, its comparison with the available FDA levels, steady-state bioaccumulation from the reference sediment, and the body burden of reference organisms. Guidance for making determinations based on these comparisons is provided in Section 7.2 and can include risk assessment and no effects levels for aquatic life.

Guidance on quality assurance/quality control (QA/QC) considerations for bioaccumulation testing are provided in Appendix G.3.17 and EPA (1995).

12.2.2 Field Assessment of Steady State Bioaccumulation

Field sampling programs obviate difficulties related to quantitatively considering field-exposure conditions in the interpretation of test results, since the animals are exposed to the conditions of mixing and sediment transport actually occurring at the disposal site. Difficulties related to the time required to conduct laboratory bioaccumulation studies are also overcome if organisms already living at the disposal site are used for field bioaccumulation studies. This approach is technically valid for predictive purposes only where there is a true historical precedent for the proposed operation being evaluated. That is, a field assessment can be used only where the quality of the sediment to be dredged can be shown not to have deteriorated

or become more contaminated since the last dredging and disposal operation. In addition, disposal has to be proposed for the site at which the dredged material in question has been previously disposed or for a site of similar sediment type supporting a similar biological community. This approach is generally not appropriate for multi-user disposal sites. Knowledge of the contaminant body burden of the organisms living around the proposed disposal site is used in evaluating bioaccumulation results in Tier IV (Section 7.2).

12.2.2.1 Apparatus

Major items required include:

- a vessel capable of operating at the disposal site and equipped to handle benthic sampling devices; navigation equipment has to allow precise positioning
- sampling devices such as a box corer, Smith-MacIntyre, Van Veen, Petersen, Ponar, Ekman or other benthic grab
- stainless steel screens to remove animals from the sediment
- tanks for transporting the animals to the laboratory in collection site water
- laboratory facilities for holding the animals prior to analysis
- chemical and analytical facilities as required for the desired analyses.

12.2.2.2 Species Selection

The species selected for analysis have to be present in sufficient numbers for adequate sample collection at all stations and to provide sufficient tissue for analysis (see Section 12.1.1). The same species must be collected at all stations because bioaccumulation cannot be compared across species lines. If these conditions cannot be met, the field assessment approach cannot be implemented.

If possible, several samples of sufficient size for analysis should be collected at each station to provide a statistical estimate of variability in tissue contaminant content. Collection of more than one sample per station, however, may prove impractical if a composite of many small organisms has to be used or if suitable organisms are not abundant at the disposal site.

To minimize the numbers and collection effort required, it is desirable to select the largest appropriate species. However, highly mobile epifauna (such as crustaceans, certain molluscs, and fish) should not be used, because a relationship cannot be established between their location when collected and their

body burden at the time of collection. Therefore, relatively large, immobile species are the most desirable organisms. However, analyses should not be conducted on single organisms as the objective is to obtain representative data for the entire population of organisms. Any relatively immobile species collectable in sufficient numbers at all stations may be used, but the required collection effort increases sharply as organism size decreases.

As discussed previously, if PAH are contaminants of concern, it is essential that bioaccumulation studies include one or more species with very low ability to metabolize PAH. Bivalve molluscs and oligochaetes are widely accepted as meeting this requirement.

12.2.2.3 Sampling Design and Conduct

Sufficient tissue to obtain definitive body burden data has to be collected using the same species from each of at least three stations within the disposal site boundaries and from an acceptable reference site. It is mandatory that several stations be sampled, rather than collecting all of the animals at one station, in order to provide a measure of the variability that exists in tissue concentrations in the animals in the area. Samples from all stations should be collected on the same day if possible.

12.2.2.4 Basis for Evaluation of Bioaccumulation

Evaluations are made by comparison to contaminant concentrations in field organisms living around, but not affected by, the disposal site, similar to the reference area approach (Section 3.1). In this case, reference data involve at least three stations located in an uncontaminated material sedimentologically similar to that within the disposal site, in a direction perpendicular to (i.e., not in the direction of) the net bottom transport. If the direction of net bottom transport is not known, at least six stations surrounding the disposal site should be established in sediments sedimentologically similar to those within the disposal site.

12.2.2.5 Sample Collection and Handling

Repeated collections should be made at the same location until an adequate tissue volume is obtained. Gently wash the sediment obtained by the sampler through 1-mm mesh stainless-steel screens, and place the retained organisms of the desired species in holding tanks.

Label the samples clearly and return the organisms to the laboratory, being careful to keep them separated and to maintain nonstressful levels of temperature and dissolved oxygen. In the laboratory, maintain them in clean water in separate containers. Do not place any sediment in the containers and do not feed the organisms. Immediately discard any organisms that die. Remove sediment from the digestive tracts of the organisms and, as possible, shells or exoskeletons (Section 12.1.2).

12.2.2.6 Chemical Analysis

Chemical analysis will involve some or all of the contaminants identified in Sections 4.2 and 9.5.1. Analytical procedures are provided in Section 9.5.2.

12.2.2.7 Data Presentation and Analysis

Complete tissue concentration data for all samples should be presented in tabular format as previously described. Since Tier IV testing will generally use non-standard methods and approaches, complete documentation is critical. Recommended statistical methods presented in Appendix D may not include all data analyses necessary for all Tier IV tests.

12.2.2.8 Conclusions

A determination is made based on the magnitude of bioaccumulation in organisms collected within the boundaries of the reference site, compared with bioaccumulation in organisms living within the area to be dredged. Guidance for making a determination based on these comparisons is provided in Section 7.2.